REMARKS

This amendment is responsive to the Office Action mailed October 31, 2007. Claims 61-64, 67, 70-73, 83-94, and 104-7 under examination in the present action. Claims 65, 66, 68, 69, 74-82 and 95-103 have been withdrawn. Claims 76, 87 and 104-107 have been cancelled.

In the Office Action mailed October 31, 2007, the Examiner objects to the claims as drawn, in part, to non-elected subject matter. Specifically, the Examiner objects to the claims because they do not specify that the essential gene is located extrachromosomally. Applicants have amended Claims 61 and 84 to specify that the functional copy of the *asd* gene is located extrachromosomally.

Additionally, the Examiner objects to Claims 88 and 91-94 because they are substantially duplicative of Claims 61 and 70-73. Claims 88 and 91-93 have been cancelled herein. Claims 89, 90, and 94 have been amended accordingly to depend from a non-cancelled claim.

Since the requested amendments relate only to requirements of the Examiner as to form, that is, to cancel substantially duplicative claims and to amend Claims 61 and 84 to be consistent with the Examiner's restriction and the elected subject matter, Applicant believes the amendments fall within the definition of amendments "complying with any requirements of form expressly set forth in a previous Office action" that are permissible under 37 C.F.R. §1.116, therefore entry and consideration of the amendments is respectfully requested.

Applicants acknowledge with appreciation the withdrawal of the rejections listed on pages 3 and 4 of the Office Action dated October 31, 2007.

Response to issues presented under 35 U.S.C. §112, second paragraph

In the Office Action, the Examiner has maintained the rejection of Claims 61, 84, 87-88 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the Examiner objects to the claim term "viable". The Examiner states:

> "Those of skill in the art know that there has been an extensive and ongoing debate for many years over what constitutes a "viable" cell [internal citations omitted]. Some equate "viability" with "culturability," while others accept that "viable but non-culturable" are alive and viable, but cannot be grown in culture. In the instant case, applicant has not stated whether a "viable" cell is one which is currently growing, or one which is capable of growing. Clearly, there is ambiguity about what this means." (Office Action dated October 31, 2007, page 5).

While it may be true there is debate regarding the term viability and/or culturability of microbial cells in general, such debate has no bearing here, particularly in regard to the Examiner restricting *asd* as the essential gene. As previously noted, the claims recite bacterial cells wherein the expression of an essential gene *asd*, which is **necessary for the production of the rigid layer of the bacterial cell wall**, is limited to permissive environmental conditions. As noted on page 20, lines 7-25 of the specification:

> "Accordingly, a preferred essential gene is *asd*, encoding β-aspartate semialdehyde dehydrogenase, **an enzyme required for the synthesis of an essential component of the rigid layer of the bacterial cell wall**, namely diaminopimelic acid (DAP). DAP is only synthesized by bacteria and is not prevalent in the environment. DAP is synthesized in six enzymatic steps from β-aspartate semialdehyde, which, in turn, is synthesized in two steps from L-aspartic acid. In the first step, L-aspartic acid is phosphorylated by one of several (usually three) β-aspartokinases which are encoded by several (usually three) separate genes regulated independently by repression and/or feedback inhibition of the gene products by the ultimate end products L-threonine, L-methionine, and L-lysine. β-aspartophosphate is converted in one step to β-aspartic semialdehyde by β-aspartic semialdehyde dehydrogenase, the product of the *asd* gene. Mutants with a point mutation in or deletion of the *asd* gene as well as mutants with mutations in any of the six genes specifying the enzymes for converting β-aspartate semialdehyde to DAP have an obligatory requirement for DAP in all media. **When DAP-requiring mutants are deprived of DAP, they die and are lysed, releasing their contents.**"

The definiteness inquiry focuses on whether *those skilled in the art* would understand the scope of the claim *when the claim is read in light of the rest of the specification.* MPEP §2173.02; *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1576, 1 USPQ 2d 1081, 1088 (1986) (emphasis added). "[T]he definiteness of the language must be analyzed--not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary level of skill in the pertinent art." *In re Moore, 439 F.2d 1235, 169 USPQ 236 (CCPA 1971).* The court in *In re Moore* further elucidated the above rule of law in a footnote, stating: "It is important here to understand that under this analysis claims which on first reading--in a vacuum, if you will--appear indefinite may *upon a reading of the specification disclosure* or prior art teachings become quite definite." *Moore,* 439 F.2d at 1235, 169 USPQ at 238 (emphasis added).

Applicants submit that the term "viable", particularly in view of the context of the claims and teachings of the specification, would be fully understood by persons skilled in the art. The bacterial cells

of the present claims cannot produce diaminopimelic acid (DAP), an essential component of the bacterial cell wall, in the non-permissive environment. A person skilled in the art would fully appreciate that the cells would be viable, that is, possess **expression of** essential genes and characteristics for cell survival and propagation, in the permissive environment and would be non-viable, that is, lack **expression of** essential genes or proteins necessary for cell survival and propagation, in the non-permissive environment.

Therefore, the specific language of Applicants' claims, especially when read in light of the specification, particularly points out and distinctly claims the subject matter of the invention. Nothing more is required of Claims 61, 84, 87-88 under 35 U.S.C. §112, second paragraph.

In view of the foregoing amendments and remarks, reconsideration and withdrawal of the rejections under 35 U.S.C. §112, second paragraph, are requested.

Response to issues presented under 35 U.S.C. §103

Galan and Guzman

In the Office Action, Claims 61-63, 67, 70-73, 83-84, 87-88, 91-94, and 104-107 continue to be rejected as obvious in view of Galan (*supra*) and Guzman et al., J. Bacteriol., 177:4121-4130 (1995)(hereinafter "Guzman").

In response to the Applicants' previous comments, the Examiner argues that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. However, Applicants point out that the Examiner cannot use the teachings of the present application to find the motivation or to cure the deficiency in each cited reference. To render obvious an invention, the prior art itself must suggest the desirability and thus, the obviousness of making the combination without the slightest recourse to the teachings of the application. Without such independent suggestion, the prior art may only be considered as inviting unguided and speculative experimentation, which is not the standard against which obviousness is determined. See e.g. Amgen, Inc. v. Chugai Pharmaceuticals Co., Ltd., 927 F.2d 1200, 18 U.S.P.Q.2d 1016 (Fed. Cir. 1991).

Applicants submit that the Examiner has failed to make a *prima facie* case of obviousness and has improperly applied hindsight reasoning in rejecting the present claims as obvious.

For example, the Examiner states:

> "Galan teaches a bacterial strain where the *asd* gene has been deleted from the bacterial chromosome and is supplied on a plasmid. Guzman teaches a promoter system that would allow expression only in the presence of arabinose (the same promoter system used by applicant in

> claim 67). Guzman further teaches that it is useful to link their promoter system to essential genes to study the depletion phenotype created by a null mutation. Therefore, one would have been motivated to link *asd* to the promoter of Guzman to study depletion phenotypes." (Office Action, page 7).

Applicants disagree. Galan merely teaches the use of "balanced-lethal" technology, which is an alternative plasmid maintenance technique to growing in the presence of antibiotics (i.e., where a plasmid-borne copy of an antibiotic resistance gene provides the means of selecting plasmid-retaining clones). The *asd* gene, essential for the viability of the cell, is deleted from the chromosome and a cloned copy is inserted into an expression vector to selectively maintain the plasmid. There is no teaching or suggestion in Galan to regulate the expression of the essential gene *asd*. In fact, in Galan, the *asd* gene is constitutively expressed in all cell environments.

Guzman teaches that the *ara*C-Pbad promoter system has tight regulation, that genes under the control of pBAD can be repressed from a 200- to 1,200-fold reduction . Guzman further teaches that this system is useful in studies to assess the effect of the expression or depletion of the gene product in mutants lacking the particular chromosomal gene.

However, there is no teaching or suggestion in either Galan or Guzman to create an environmentally limited viability system wherein a cell is viable in one environment and not viable in another. As noted in Applicants' specification, this discovery is extremely useful for vaccine microorganisms, where oral administration of live avirulent vaccine microorganisms can lead to fecal shedding of the recombinant microorganisms, with the potential risk that the bacterial vaccine strain will proliferate in nature and infect individuals not selected for immunization.

The Examiner's generalization that one of skill in the art would have been motivated, without the knowledge or teaching of Applicants' specification, to "link *asd* to the promoter of Guzman to study depletion phenotypes" fails to establish *prima facie* obviousness and is pure hindsight reasoning. According to the Examiner, *asd* and its function and its use in complementation was known from Galan; what further study was suggested by combining Guzman with Galan? According to the Examiner's characterization of Galan, the affects of *asd* depletion phenotypes were known to those skilled in the art and therefore persons skilled in the art would not have been motivated to "express a cloned gene from an inducible promoter and assess the effect of the expression or depletion of the gene product in mutants lacking the chromosomal gene" as taught by Guzman (Guzman et al., page 4121).

In reality, the Examiner is conducting improper hindsight reconstruction of the invention using Applicants' specification as a guide to pick out individual components of the invention that were known in the art. The elegantly simple nature of Applicants' invention, once disclosed, leads the Examiner to

select individual components of the invention and make bald conclusory statements regarding motivation, e.g., further general scientific study, without finding that motivation in the references themselves.

However, Applicants' combination and application of components, some of which individual components were known at the time of filing, to create a new and useful environmentally limited viability system to further promote the field of microbial vaccines, are neither taught nor suggested in any of the cited references.

As previously, noted, the disclosure, in its broadest sense (i.e., not limited to the current restricted embodiment), relates to environmentally limited viability systems (ELVS) for microbes based on differences in environmental conditions, i.e., permissive and non-permissive environments. Viability of the microorganisms is limited to the permissive environment by specifically expressing one or more genes essential to cell viability only while in the permissive environment, and/or expressing one or more lethal genes only in the non-permissive environment. (*See, e.g.*, page 6, lines 1-12 of the specification.) None of the cited references includes the concept of permissive and non-permissive environments paired to a particular chromosomal deletion and regulated expression of a non-chromosomal essential genetic element.

Accordingly, because neither Galan nor Guzman, alone or in combination, teach or suggest controlling the viability of a bacterial cell in defined permissive and non-permissive environments, the claims cannot be obvious as a matter of law. Reconsideration and removal of the rejection of Claims 61-63, 67, 70-73, 83-84, 87-88, 91-94, and 104-107 are requested.

Galan and Glick

In the Office Action, Claims 61-62, 64, 70-73, 83-86, 87-94, and 104-107 continue to be rejected as obvious in view of Galan (*see, supra*) and Glick et al., Molecular Biotechnology, Principles and Applications of Recombinant DNA, (1994) ASM press, pp. 90-92) (hereinafter "Glick"). The Examiner states:

> "Therefore, it would have been obvious to one of skill in the art to use the temperature regulated *p*L promoter system (as disclosed by Glick *et al.*) to control expression of the *asd* gene in the bacterial cells of Galan *et al.* because regulatable strong promoters are advantageous to avoid a high level of continual expression of a cloned gene which is often detrimental to the host cell." (Office Action, page 19).

Once again, for the same reasons as discussed above, Applicants disagree. The deficiencies of Galan have been previously discussed. Galan merely teaches the use of "balanced-lethal" technology,

which is an alternative plasmid maintenance technique to growing in the presence of antibiotics (i.e., with a plasmid-borne copy of an antibiotic resistance gene). There is no teaching or suggestion in Galan to regulate the expression of the essential gene *asd*. In fact, in Galan, the *asd* gene is constitutively expressed in all cell environments.

Glick teaches the CI857/pL promoter system, noting that this system can be used for temperature-regulated transcription of genes operably linked to the pL promoter.

However, there is no teaching or suggestion in either Galan or Glick or their combination to create an environmentally limited viability system wherein a cell is viable in one environment and not viable in another. As noted in Applicants' specification, this discovery is extremely useful for vaccine microorganisms, where oral administration of live avirulent vaccine microorganisms can lead to fecal shedding of the microorganisms, with the potential risk that the bacterial vaccine strain will proliferate in nature and infect individuals not selected for immunization.

Once again, the Examiner is merely applying hindsight reconstruction and making bald general statements regarding the motivation to combine the references. In the present case, the Examiner contends:

> "Glick teaches the use of a temperature sensitive promoter (the same promoter disclosed by applicant in the instant specification). Glick further teaches that this promoter is useful in controlling transcription to avoid a high level of continual expression which is often detrimental too a host cell. **Therefore, one would have been motivated to link *asd* to the promoter of Glick to control detrimental high-level transcription.**" (Office Action, page 10.)

Once again, Applicants question the Examiner's suggestion that high levels of *asd* expression is a problem in the art, or alternatively, if true, would lead persons skilled in the art to link *asd* to the temperature sensitive promoter of Glick. Galan teaches operatively linking the *asd* gene to the strong recombinant constitutive promoter P_{trc}, yet Galan fails to mention any problem or difficulty with high level expression. To the contrary, Galan notes the cells were stable and had normal growth. Accordingly, Applicants submit the Examiner has failed to establish a *prima facie* case of obviousness and is merely using improper hindsight reconstruction of the invention and subtituting conclusory statements for the necessary evidence in the art of motivation on the part of a person of ordinary skill leading to a suggestion or approximation the claimed invention.

Accordingly, because neither Galan nor Glick, alone or in combination, teach or suggest controlling the viability of a bacterial cell in permissive and non-permissive environments, the claims

cannot be obvious as a matter of law. Reconsideration and removal of the rejection of Claims 61-63, 67, 70-73, 83-84, 87-88, 91-94, and 104-107 are requested.

Curtiss '483, Guzman, Curtiss '345, and Glick

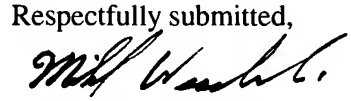
In the Office Action, Claims 61-62, 64, 70-73, 83-86, 87-94, and 104-107 have additionally been rejected as obvious in view of various combinations of Curtiss '483, Guzman, Curtiss '345 and Glick. The deficiencies of all these references with respect to the present invention have been addressed above. As noted previously, both Curtiss references refer to plasmid maintenance systems which ensure that an antigen-encoding vector will be maintained in the population of bacterial cells intended as a vaccine. The present claims utilize an environment-triggered cell death to ensure that a recombinant vaccine strain cannot survive in particular environments (e.g., outside the vaccinated host), thus achieving a biological containment system. It is clear that the "balanced-lethal" technology does not and cannot accomplish effective biological containment, because the vaccine cells survive in and out of the host due to the presence of the essential gene on the plasmid, which gene is constitutively expressed.

There is no teaching or suggestion in any of Curtiss '483, Guzman, Curtiss '345, or Glick to create an environmentally limited viability system wherein a cell is viable in one environment and not viable in another.

MPEP §2143.03 states that "[t]o establish *prima facie* obviousness of a claimed invention, all claim limitations must be taught or suggested by the prior art." (emphasis added) In the present case, critical limitations and recitation of the present claims are only taught by Applicants' specification (and not by any of the cited prior art).

Accordingly, because none of the citations, alone or in combination, teach or suggest controlling the viability of a bacterial cell to permissive and non-permissive environments, the claims cannot be obvious as a matter of law. Reconsideration and removal of the rejection of Claims 61-63, 67, 70-73, 83-84, 87-88, 91-94, and 104-107 are requested.

Respectfully submitted,



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